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Litton

Mutagenic evaluation of compound 000128370 Butylated Hydroxytoluene (Ionol)
(71-25) 4/30/75

MUTAGENIC EVALUATION OF
COMPOUND 000128370
BUTYLATED HYDROXYTOLUENE
(IONOL)
(71-25)

5516 Nicholson Lane
Kensington, Maryland
20795

LBI PROJECT #2468

MUTAGENIC EVALUATION OF
COMPOUND 000128370
BUTYLATED HYDROXYTOLUENE
(IONOL)
(71-25)

SUBMITTED TO
FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

APRIL 30, 1975



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EVALUATION SUMMARY

Compound 000128370, Butylated Hydroxytoluene, did not exhibit genetic activity in any of the assays employed in this evaluation.



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DATE: April 30, 1975

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound 000128370, Butylated Hydroxytoluene
(Iono1)

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974
2. Description: White, crystalline material

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains: TA-1535
TA-1537
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

| <u>Component</u> | <u>Final Concentration/ml</u> | |
|-------------------------------|-------------------------------|----|
| 1. TPN (sodium salt) | 6 | μM |
| 2. Isocitric acid | 49 | μM |
| 3. Tris buffer, pH 7.4 | 28 | μM |
| 4. MgCl ₂ | 1.7 | μM |
| 5. Tissue homogenate fraction | 72 | mg |



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D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

| <u>Assay</u> | <u>Chemical</u> ^a | <u>Solvent</u> | <u>Probable Mutagenic Specificity</u> |
|----------------|------------------------------|--------------------------------|---|
| Non-activation | Ethyl methanesulfonate | Water or saline | BPS |
| | 2-Nitrofluorene | Dimethylsulfoxide ^c | FS |
| | Quinacrine mustard | Water or saline | FS |
| Activation | Dimethylnitrosamine | Water or saline | BPS |
| | 2-Acetylaminofluorene | Dimethylsulfoxide ^c | FS |

^a Concentrations given in the Results Section

^b BPS = base-pair substitution; FS = frameshift

^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

In the nonactivation procedure, approximately 10^9 cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.



D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.



IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound:
000128370 Butylated Hydroxytoluene (Ionol)
2. Test solvent: DMSO
3. Solubility of the test compound under treatment conditions:
Soluble under treatment conditions.
4. Additional comments: white, crystalline material

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: November 13, 1974
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

| <u>Dose</u> | <u>Percent Concentration</u> | |
|------------------|------------------------------|--------------|
| | <u>Bacteria</u> | <u>Yeast</u> |
| 1/4 50% Survival | 0.15 | 0.6 |
| 1/2 50% Survival | 0.3 | 1.2 |
| 50% Survival | 0.6 | 2.4 |
| Plate Tests | 0.3 | -- |



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IV. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: 000128370

B. Test date: April 23, 1975

C. Concentration of the test compound: 0.3%

| Test | Species | Tissue | TA-1535 | | TA-1537 | | TA-1538 | | |
|-------------------------------|---------|--------|------------------|------------------|----------|----------|----------|----------|----------|
| | | | <u>1</u> | <u>2</u> | <u>1</u> | <u>2</u> | <u>1</u> | <u>2</u> | |
| 1. <u>Non-activation</u> | | | | | | | | | |
| Solvent Control | --- | --- | 138 | 146 | 25 | 22 | 26 | 35 | |
| Positive Control ^a | --- | --- | >10 ⁴ | >10 ⁴ | 195 | 176 | 158 | 219 | |
| Test Compound | --- | --- | 169 | 179 | 15 | 15 | 37 | 30 | |
| 2. <u>Activation</u> | | | | | | | | | |
| Negative Control | --- | --- | 16 | 15 | 27 | 33 | 11 | 14 | |
| Solvent Control | --- | --- | 12 | 9 | 36 | 43 | 17 | 18 | |
| Reaction Mixture Control | --- | --- | 10 | 18 | 36 | 39 | 9 | 17 | |
| Positive Control ^b | Mouse | Liver | >10 ³ | >10 ³ | 146 | 143 | 239 | 225 | |
| Positive Control | | Lung | 9 | 8 | 33 | 33 | 14 | 11 | |
| Positive Control | | Testes | 11 | 7 | 37 | 32 | 15 | 15 | |
| Positive Control | Rat | Liver | >10 ³ | >10 ³ | 84 | 80 | 329 | 313 | |
| Positive Control | | Lung | 10 | 8 | 32 | 35 | 16 | 12 | |
| Positive Control | | Testes | 11 | 6 | 24 | 43 | 16 | 16 | |
| Positive Control | Monkey | Liver | >10 ³ | >10 ³ | 47 | 43 | 122 | 129 | |
| Positive Control | | Lung | 7 | 9 | 32 | 38 | 15 | 10 | |
| Positive Control | | Testes | 9 | 5 | 28 | 33 | 15 | 15 | |
| Test Compound | Mouse | Liver | 9 | 15 | 55 | 65 | 12 | 20 | |
| Test Compound | | Lung | 9 | 7 | 33 | 18 | 11 | 11 | |
| Test Compound | | Testes | 6 | 10 | 28 | 36 | 18 | 13 | |
| Test Compound | Rat | Liver | 7 | 12 | 62 | 51 | 12 | 20 | |
| Test Compound | | Lung | 7 | 9 | 27 | 25 | 21 | 14 | |
| Test Compound | | Testes | 6 | 5 | 20 | 31 | 18 | 11 | |
| Test Compound | Monkey | Liver | 8 | 11 | 55 | 51 | 13 | 20 | |
| Test Compound | | Lung | 5 | 7 | 25 | 22 | 12 | 10 | |
| Test Compound | | Testes | 3 | 2 | 18 | 32 | 16 | 8 | |
| a | TA-1535 | EMS | 10 | μl/plate | b | TA-1535 | DMNA | 50 | μm/plate |
| | TA-1537 | QM | 20 | μg/plate | | TA-1537 | AAF | 100 | μg/plate |
| | TA-1538 | NF | 100 | μg/plate | | TA-1538 | AAF | 100 | μg/plate |



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DATA TABLE TERMS AND ABBREVIATIONS

| ABBREVIATION OR TERM | DEFINITION OR EXPLANATION |
|-------------------------|---|
| COMPOUND | Client designated compound number appears in this column. |
| TEST CODES | <p> NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) </p> <p> A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound A+T = Activation: Tissue Control </p> <p> LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels </p> |
| CONCENTRATION | <p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p> |
| POPU | Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $X 10^6$). |
| MUT 1 | Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $X 10^0$). For strain D4, MUT 1 represents the number of ADE+ convertants. |
| MUT 2 | Only used for strain D4 and represents the number of TRY+ convertants in the plated sample. |
| FREQ 1 | The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value. |
| FREQ 2 | Only used for strain D4 and represents the TRY+ conversion frequency. |
| CONTAM | Presence of contamination on any plates. |



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DATA TABLE TERMS AND ABBREVIATIONS (continued)

| ABBREVIATION OR TERM | DEFINITION OR EXPLANATION |
|-------------------------|---|
| AAF | 2-Acetylaminofluorene |
| DMSO | Dimethylsulfoxide |
| DMN | Dimethylnitrosamine |
| EMS | Ethyl Methanesulfonate |
| QM | Quinacrine Mustard |
| NF | Nitrofluorene |
| SPECIES | Animal Strains |
| SPRDAW | Sprague Dawley Rats |
| ICRFLO | Flow ICR Random Bred Mice |
| RHESUS | Rhesus Monkey (<u>Macaca mulatta</u>) |
| MIXEDB | Dog, Mixed Breed |
| NEWZEA | New Zealand White Rabbit |



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES

COMPOUND 000128370

| TEST | ORG | TA1535 HIS EX-8 | TA1537 HIS EX-8 | TA1538 HIS EX-8 | 000004 ADE EX-5 | 000004 TRY EX-5 |
|------|-----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| NAN | | 1.22 | 4.28 | 9.79 | 4.37 | 4.76 |
| NAP | | 1088.21 | 415.68 | 181.23 | 160.96 | 60.50 |
| NA1 | | 3.72 | 4.27 | 7.51 | 2.76 | 2.87 |
| NA2 | | 2.30 | 4.60 | 7.71 | 3.70 | 2.90 |

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES ICRFLO COMPOUND 000128370

| TEST | ORG | TA1535 HIS EX-8 | TA1537 HIS EX-8 | TA1538 HIS EX-8 | 0000D4 ADE EX-5 | 0000D4 TRY EX-5 |
|------|-----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| ACT | A+C | 2.63 | 3.70 | 4.95 | 3.56 | 4.07 |
| ACT | A-C | 1.05 | 3.44 | 2.56 | 3.82 | 6.21 |
| ACT | PLI | 58.39 | 14.57 | 36.50 | 7.31 | 9.96 |
| ACT | PLU | 1.15 | 3.31 | 4.31 | 3.71 | 3.84 |
| ACT | PTE | 9.55 | 3.74 | 3.35 | 3.06 | 6.12 |
| ACT | LI1 | 1.53 | 2.30 | 1.89 | 3.05 | 5.24 |
| ACT | LI2 | 1.33 | 3.21 | 3.47 | 4.39 | 4.23 |
| ACT | LU1 | 0.99 | 2.26 | 1.97 | 4.66 | 4.23 |
| ACT | LU2 | 1.58 | 2.64 | 2.70 | 3.89 | 4.03 |
| ACT | TE1 | 2.18 | 4.00 | 2.39 | 5.12 | 5.43 |
| ACT | TE2 | 1.66 | 4.18 | 2.87 | 3.57 | 6.08 |

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES SPRDAW COMPOUND 000128370

| TEST | ORG | TA1535 HIS EX-8 | TA1537 HIS EX-8 | TA1538 HIS EX-8 | 0000D4 ADE EX-5 | 0000D4 TRY EX-5 |
|------|-----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| ACT | A+C | 3.12 | 10.83 | 10.78 | 2.69 | 2.22 |
| ACT | A-C | 4.26 | 8.33 | 5.64 | 4.67 | 3.00 |
| ACT | PLI | 49.05 | 18.14 | 129.82 | 6.49 | 4.25 |
| ACT | PLU | 2.70 | 5.39 | 15.38 | 4.51 | 4.26 |
| ACT | PTE | 3.52 | 12.52 | 6.04 | 2.91 | 4.07 |
| ACT | LI1 | 3.76 | 6.49 | 3.89 | 3.36 | 3.78 |
| ACT | LI2 | 3.56 | 7.36 | 6.09 | 4.03 | 3.56 |
| ACT | LU1 | 1.93 | 4.55 | 4.75 | 3.17 | 5.49 |
| ACT | LU2 | 3.41 | 3.45 | 6.48 | 2.41 | 3.02 |
| ACT | TE1 | 3.93 | 11.41 | 6.37 | 3.65 | 4.61 |
| ACT | TE2 | 2.33 | 7.56 | 5.78 | 2.53 | 3.38 |

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES RHESUS COMPOUND 000128370

| TEST | ORG | TA1535 HIS EX-8 | TA1537 HIS EX-8 | TA1538 HIS EX-8 | TA1538 HIS EX-8 | 000004 ADE EX-5 | 000004 TRY EX-5 |
|------|-----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| ACT | A+C | 5.36 | 5.04 | 15.13 | | 5.59 | 4.93 |
| ACT | A-C | 8.28 | 3.26 | 4.81 | 14.06 | 5.31 | 6.15 |
| ACT | PLI | 197.63 | 14.44 | 49.46 | | 5.89 | 6.26 |
| ACT | PLU | 15.17 | 2.95 | 4.60 | | 3.75 | 5.23 |
| ACT | PTE | 5.03 | 5.26 | 5.69 | | 4.84 | 3.49 |
| ACT | LI1 | 2.31 | 6.12 | 14.29 | 3.93 | 4.13 | 5.21 |
| ACT | LI2 | 8.22 | 4.80 | 140.00 | 2.27 | 2.59 | 4.92 |
| ACT | LU1 | 3.89 | 6.02 | 5.43 | 2.49 | 5.11 | 5.11 |
| ACT | LU2 | 6.59 | 3.26 | 3.77 | 6.24 | 3.49 | 3.37 |
| ACT | TE1 | 4.32 | 6.26 | 1.79 | | 2.88 | 4.74 |
| ACT | TE2 | 9.13 | 3.55 | 2.31 | | 1.78 | 0.00 |

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound 000128370, Butylated Hydroxytoluene, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate Tests

At a concentration of 0.3%, 000128370 was not mutagenic for any of the bacteria indicator strains employed in either direct or activation plate tests.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The Rhesus LI1 and LI2 doses with TA-1538 were repeated because the initial assays exhibited high reversion frequencies due to defective population plates. The repeats were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

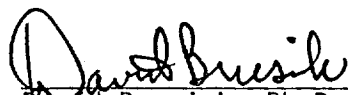
2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

Compound 000128370 did not exhibit genetic activity in any of the in vitro microbial assays employed in this evaluation.

Submitted by:


David Brusick, Ph.D.
Director of Genetics



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APPENDIX
Tabulation of Data



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 431701

DETECTOR TA1535

SPECIES

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | NAN | | SALINE | 0576 | 0007 | 1.22 | 2 |
| | NAP | | EMS 0.002 % | 0390 | 4244 | 1088.21 | 0 |
| 000128370 | NA1 | | 0003-1 PCT. | 0296 | 0011 | 3.72 | 0 |
| 000128370 | NA2 | | 0015-2 PCT. | 0479 | 0011 | 2.30 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

| | | CONTRACT 22374-2104 | | PROJECT 02468 | | | |
|-------------------|------|---------------------|---------------|-----------------|-----------|------------|--------|
| EXPERIMENT 431702 | | DETECTOR TA1537 | | SPECIES | | | |
| | | | | DATE - 04/25/75 | | | |
| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
| | NAN | | SALINE | 0817 | 0035 | 4.28 | 0 |
| | NAP | | QM 1.0 UG/ML | 0236 | 0981 | 415.68 | 0 |
| 000128370 | NA1 | | 0003-1 PCT. | 0866 | 0037 | 4.27 | 0 |
| 000128370 | NA2 | | 0015-2 PCT. | 0783 | 0036 | 4.60 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

| | | | | | | | |
|-------------------|------|---------------------|---------------|-----------------|-----------|------------|--------|
| | | CONTRACT 22374-2104 | | PROJECT 02468 | | | |
| EXPERIMENT 431703 | | DETECTOR TA1538 | | SPECIES | | | |
| | | | | DATE - 04/25/75 | | | |
| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
| | NAN | | DMSO | 0337 | 0033 | 9.79 | 0 |
| | NAP | | NF 125 UG-ML | 0341 | 0618 | 181.23 | 0 |
| 000128370 | NA1 | | 0003-1 PCT. | 0333 | 0025 | 7.51 | 0 |
| 000128370 | NA2 | | 0015-2 PCT. | 0376 | 0029 | 7.71 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 433801

DETECTOR 0000D4

SPECIES

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+4 | MUT1 EP+1 | MUT2 EP+1 | FREQ1 EP-5 | FREQ2 EP-5 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|--------------|---------------|---------------|--------|
| | NAN | | SALINE | 1303 | 0057 | 0062 | 4.37 | 4.76 | 0 |
| | NAP | | EMS 1.0 % | 0643 | 1035 | 0389 | 160.96 | 60.50 | 0 |
| 000128370 | NA1 | | 0012-1 PCT. | 0870 | 0024 | 0025 | 2.76 | 2.87 | 0 |
| 000128370 | NA2 | | 0006-1 PCT. | 1000 | 0037 | 0029 | 3.70 | 2.90 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 432301 DETECTOR TA1535 SPECIES ICRFLO DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | DMN 50 UM/ML | 0799 | 0021 | 2.63 | 0 |
| | A-C | | SALINE | 0667 | 0007 | 1.05 | 0 |
| | ACP | LI | DMN 50 UM/ML | 0793 | 0463 | 58.39 | 0 |
| | ACP | LU | DMN 50 UM/ML | 0695 | 0008 | 1.15 | 2 |
| | ACP | TE | DMN 50 UM/ML | 0555 | 0053 | 9.55 | 2 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0652 | 0010 | 1.53 | 2 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0679 | 0009 | 1.33 | 2 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0505 | 0005 | 0.99 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0568 | 0009 | 1.58 | 2 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0551 | 0012 | 2.18 | 2 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0602 | 0010 | 1.66 | 2 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 432401

DETECTOR TA1537

SPECIES ICRFLO

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 1677 | 0062 | 3.70 | 0 |
| | A-C | | DMSO | 1365 | 0047 | 3.44 | 0 |
| | ACP | LI | AAF 800 UG/ML | 1119 | 0163 | 14.57 | 0 |
| | ACP | LU | AAF 800 UG/ML | 1180 | 0039 | 3.31 | 2 |
| | ACP | TE | AAF 800 UG/ML | 1417 | 0053 | 3.74 | 2 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 1437 | 0033 | 2.30 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 1432 | 0046 | 3.21 | 2 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 1241 | 0028 | 2.26 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 1212 | 0032 | 2.64 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 1201 | 0048 | 4.00 | 2 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 1172 | 0049 | 4.18 | 2 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 432501

DETECTOR TA1538

SPECIES ICRFLD

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 0646 | 0032 | 4.95 | 0 |
| | A-C | | DMSO | 0742 | 0019 | 2.56 | 0 |
| | ACP | LI | AAF 800 UG/ML | 0737 | 0269 | 36.50 | 2 |
| | ACP | LU | AAF 800 UG/ML | 0510 | 0022 | 4.31 | 0 |
| | ACP | TE | AAF 800 UG/ML | 0717 | 0024 | 3.35 | 2 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0845 | 0016 | 1.89 | 2 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0720 | 0025 | 3.47 | 2 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0660 | 0013 | 1.97 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0481 | 0013 | 2.70 | 2 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0628 | 0015 | 2.39 | 2 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0627 | 0018 | 2.87 | 2 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

| CONTRACT 22374-2104 | | | | PROJECT 02468 | | | | | |
|---------------------|------|-----------------|---------------|----------------|--------------|--------------|-----------------|---------------|--------|
| EXPERIMENT 433701 | | DETECTOR 000004 | | SPECIES ICRFL0 | | | DATE - 04/25/75 | | |
| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+4 | MUT1 EP+1 | MUT2 EP+1 | FREQ1 EP-5 | FREQ2 EP-5 | CONTAM |
| | A+C | | DMN 90 UM/ML | 0787 | 0028 | 0032 | 3.56 | 4.07 | 6 |
| | A-C | | SALINE | 0837 | 0032 | 0052 | 3.82 | 6.21 | 0 |
| | ACP | LI | DMN 90 UM/ML | 0793 | 0058 | 0079 | 7.31 | 9.96 | 0 |
| | ACP | LU | DMN 90 UM/ML | 0781 | 0029 | 0030 | 3.71 | 3.84 | 0 |
| | ACP | TE | DMN 90 UM/ML | 0556 | 0017 | 0034 | 3.06 | 6.12 | 6 |
| 000128370 | ACT | LI1 | 0012-1 PCT. | 0820 | 0025 | 0043 | 3.05 | 5.24 | 4 |
| 000128370 | ACT | LI2 | 0006-1 PCT. | 0615 | 0027 | 0026 | 4.39 | 4.23 | 0 |
| 000128370 | ACT | LU1 | 0012-1 PCT. | 0686 | 0032 | 0029 | 4.66 | 4.23 | 6 |
| 000128370 | ACT | LU2 | 0006-1 PCT. | 0694 | 0027 | 0028 | 3.89 | 4.03 | 4 |
| 000128370 | ACT | TE1 | 0012-1 PCT. | 0645 | 0033 | 0035 | 5.12 | 5.43 | 4 |
| 000128370 | ACT | TE2 | 0006-1 PCT. | 0756 | 0027 | 0046 | 3.57 | 6.08 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 431801

DETECTOR TA1535

SPECIES SPRDAW

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | DMN 50 UM/ML | 0513 | 0016 | 3.12 | 0 |
| | A-C | | SALINE | 0470 | 0020 | 4.26 | 0 |
| | ACP | LI | DMN 50 UM/ML | 0422 | 0207 | 49.05 | 0 |
| | ACP | LU | DMN 50 UM/ML | 0370 | 0010 | 2.70 | 0 |
| | ACP | TE | DMN 50 UM/ML | 0227 | 0008 | 3.52 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0425 | 0016 | 3.76 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0533 | 0019 | 3.56 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0363 | 0007 | 1.93 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0323 | 0011 | 3.41 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0305 | 0012 | 3.93 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0300 | 0007 | 2.33 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 431901 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 FP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 0600 | 0065 | 10.83 | 0 |
| | A-C | | DMSO | 0840 | 0070 | 8.33 | 0 |
| | ACP | LI | AAF 800 UG/ML | 0998 | 0181 | 18.14 | 0 |
| | ACP | LU | AAF 800 UG/ML | 1169 | 0063 | 5.39 | 0 |
| | ACP | TE | AAF 800 UG/ML | 0735 | 0092 | 12.52 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0940 | 0061 | 6.49 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0856 | 0063 | 7.36 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 1034 | 0047 | 4.55 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0870 | 0030 | 3.45 | 2 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0754 | 0086 | 11.41 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0714 | 0054 | 7.56 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 432201 DETECTOR TA1538 SPECIES SPRDAW DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 0371 | 0040 | 10.78 | 0 |
| | A-C | | DMSO | 0443 | 0025 | 5.64 | 0 |
| | ACP | LI | AAF 800 UG/ML | 0228 | 0296 | 129.82 | 0 |
| | ACP | LU | AAF 800 UG/ML | 0312 | 0048 | 15.38 | 0 |
| | ACP | TE | AAF 800 UG/ML | 0298 | 0018 | 6.04 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0257 | 0010 | 3.89 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0279 | 0017 | 6.09 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0295 | 0014 | 4.75 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0293 | 0019 | 6.48 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0267 | 0017 | 6.37 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0329 | 0019 | 5.78 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 434301 DETECTOR 000004 SPECIES SPRDAW DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+4 | MUT1 EP+1 | MUT2 EP+1 | FREQ1 FP-5 | FREQ2 FP-5 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|--------------|---------------|---------------|--------|
| | A+C | | DMN 90 UM/ML | 0854 | 0023 | 0019 | 2.69 | 2.22 | 0 |
| | A-C | | SALINE | 1135 | 0053 | 0034 | 4.67 | 3.00 | 0 |
| | ACP | LI | DMN 90 UM/ML | 0848 | 0055 | 0036 | 6.49 | 4.25 | 0 |
| | ACP | LU | DMN 90 UM/ML | 0821 | 0037 | 0035 | 4.51 | 4.26 | 0 |
| | ACP | TE | DMN 90 UM/ML | 0860 | 0025 | 0035 | 2.91 | 4.07 | 0 |
| 000128370 | ACT | LI1 | 0012-1 PCT. | 0952 | 0032 | 0036 | 3.36 | 3.78 | 0 |
| 000128370 | ACT | LI2 | 0006-1 PCT. | 0843 | 0034 | 0030 | 4.03 | 3.56 | 0 |
| 000128370 | ACT | LU1 | 0012-1 PCT. | 0820 | 0026 | 0045 | 3.17 | 5.49 | 0 |
| 000128370 | ACT | LU2 | 0006-1 PCT. | 0995 | 0024 | 0030 | 2.41 | 3.02 | 0 |
| 000128370 | ACT | TE1 | 0012-1 PCT. | 0932 | 0034 | 0043 | 3.65 | 4.61 | 6 |
| 000128370 | ACT | TE2 | 0006-1 PCT. | 0829 | 0021 | 0028 | 2.53 | 3.38 | 0 |



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REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

| | | CONTRACT 22374-2104 | | PROJECT 02468 | | | |
|-------------------|------|---------------------|---------------|----------------|-----------|-----------------|--------|
| EXPERIMENT 432604 | | DETECTOR TA1535 | | SPECIES RHESUS | | DATE - 04/25/75 | |
| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
| | A+C | | DMN 50 UM/ML | 0392 | 0021 | 5.36 | 0 |
| | A-C | | SALINE | 0338 | 0028 | 8.28 | 0 |
| | ACP | LI | DMN 50 UM/ML | 0337 | 0666 | 197.63 | 0 |
| | ACP | LU | DMN 50 UM/ML | 0211 | 0032 | 15.17 | 0 |
| | ACP | TE | DMN 50 UM/ML | 0298 | 0015 | 5.03 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0260 | 0006 | 2.31 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0073 | 0006 | 8.22 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0283 | 0011 | 3.89 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0334 | 0022 | 6.59 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0324 | 0014 | 4.32 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0252 | 0023 | 9.13 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 433101

DETECTOR TA1537

SPECIES RHESUS

DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 0794 | 0040 | 5.04 | 0 |
| | A-C | | DMSO | 1135 | 0037 | 3.26 | 0 |
| | ACP | LI | AAF 800 UG/ML | 1122 | 0162 | 14.44 | 0 |
| | ACP | LU | AAF 800 UG/ML | 0848 | 0025 | 2.95 | 0 |
| | ACP | TE | AAF 800 UG/ML | 0874 | 0046 | 5.26 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0719 | 0044 | 6.12 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0667 | 0032 | 4.80 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0714 | 0043 | 6.02 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0827 | 0027 | 3.26 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0767 | 0048 | 6.26 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0959 | 0034 | 3.55 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 433601 DETECTOR TA1538 SPECIES RHESUS DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPUI EP+6 | MUT1 EP+0 | FREQ1 FP-8 | CONTAM |
|-----------|------|-----------|---------------|---------------|--------------|---------------|--------|
| | A+C | | AAF 800 UG/ML | 0152 | 0023 | 15.13 | 0 |
| | A-C | | DMSO | 0187 | 0009 | 4.81 | 0 |
| | ACP | LI | AAF 800 UG/ML | 0279 | 0138 | 49.46 | 0 |
| | ACP | LU | AAF 800 UG/ML | 0261 | 0012 | 4.60 | 1 |
| | ACP | TE | AAF 800 UG/ML | 0246 | 0014 | 5.69 | 0 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0063 | 0009 | 14.29 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0005 | 0007 | 140.00 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0092 | 0005 | 5.43 | 0 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0053 | 0002 | 3.77 | 0 |
| 000128370 | ACT | TE1 | 0003-1 PCT. | 0392 | 0007 | 1.79 | 0 |
| 000128370 | ACT | TE2 | 0015-2 PCT. | 0390 | 0009 | 2.31 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 434501 DETECTOR TA1538 SPECIES RHESUS DATE - 04/25/75

| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+6 | MUT1 EP+0 | FREQ1 EP-8 | CONTAM |
|-----------|------|-----------|---------------|--------------|--------------|---------------|--------|
| | A-C | | DMSO | 0576 | 0081 | 14.06 | 1 |
| 000128370 | ACT | LI1 | 0003-1 PCT. | 0484 | 0019 | 3.93 | 0 |
| 000128370 | ACT | LI2 | 0015-2 PCT. | 0528 | 0012 | 2.27 | 0 |
| 000128370 | ACT | LU1 | 0003-1 PCT. | 0523 | 0013 | 2.49 | 2 |
| 000128370 | ACT | LU2 | 0015-2 PCT. | 0545 | 0034 | 6.24 | 0 |



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

| CONTRACT 22374-2104 | | | | PROJECT 02468 | | | | | |
|---------------------|------|-----------------|---------------|----------------|-----------|-----------------|------------|------------|--------|
| EXPERIMENT 434401 | | DETECTOR 0000D4 | | SPECIES RHESUS | | DATE - 04/25/75 | | | |
| COMPOUND | TEST | ORG ID | CONCENTRATION | POPU EP+4 | MUT1 EP+1 | MUT2 EP+1 | FREQ1 FP-5 | FREQ2 EP-5 | CONTAM |
| | A+C | | DMN 90 UM/ML | 0912 | 0051 | 0045 | 5.59 | 4.93 | 4 |
| | A-C | | SALINE | 0960 | 0051 | 0059 | 5.31 | 6.15 | 4 |
| | ACP | LI | DMN 90 UM/ML | 0815 | 0048 | 0051 | 5.89 | 6.26 | 4 |
| | ACP | LU | DMN 90 UM/ML | 0879 | 0033 | 0046 | 3.75 | 5.23 | 4 |
| | ACP | TE | DMN 90 UM/ML | 0888 | 0043 | 0031 | 4.84 | 3.49 | 4 |
| 000128370 | ACT | LI1 | 0012-1 PCT. | 0921 | 0038 | 0048 | 4.13 | 5.21 | 6 |
| 000128370 | ACT | LI2 | 0006-1 PCT. | 0772 | 0020 | 0038 | 2.59 | 4.92 | 0 |
| 000128370 | ACT | LU1 | 0012-1 PCT. | 0763 | 0039 | 0039 | 5.11 | 5.11 | 4 |
| 000128370 | ACT | LU2 | 0006-1 PCT. | 0802 | 0028 | 0027 | 3.49 | 3.37 | 4 |
| 000128370 | ACT | TE1 | 0012-1 PCT. | 1077 | 0031 | 0051 | 2.88 | 4.74 | 0 |
| 000128370 | ACT | TE2 | 0006-1 PCT. | 0676 | 0012 | 0000 | 1.78 | 0.00 | 6 |